RESET06 (AT15-6) Microbiology Team Report

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In general terms, we are interested in studying the response of the microbiological communities to the recent eruption at 9˚N. We are particularly interested in chemoautotrophic microorganisms, which form the basis of deep-sea hydrothermal vent ecosystems. Never is this more apparent than after an eruption, which basically wipes out the lush animal communities typically associated with these systems. Thus, the recent eruption represents a unique opportunity to study the microbial communities after a major disturbance and to follow their succession through time.
During this cruise we investigated the microbial communities in three main habitats: a) sulfide chimneys, b) surfaces of newly exposed basalt, and c) fluids emanating from diffuse flow vents. The obtained samples were shared between the laboratories of Sievert/Taylor/Summons and Vetriani, and subsequent analyses will be undertaken that complement each other. The data obtained in this cruise will serve as a benchmark for future cruises and analyses to document the changes in the microbial communities occurring over time. In the following, we have listed the goals, samples taken, and preliminary results of the two groups (Vetriani and Sievert/Taylor/Summons) separately.

Vetriani, Voordeckers (Rutgers University)

The goals for this cruise were: 1) Obtain samples of hydrothermal sulfides and fluids for a) inoculation of cultures both aboard ship and at Rutgers University laboratories to isolate, identify, and characterize novel microorganisms, hydrogen oxidizers/nitrate reducers (anaerobic) and thiosulfate oxidizers (aerobic and anaerobic) and MPN work and b) survey of functional genes (nitrate reducers and for methanogens/methanotrophs) and RNA transcripts (nitrate reduction genes). 2) Deployment of stainless steel mesh cultivators in diffuse flows in order to promote the growth of biomass for later retrieval and study.

Samples

Sulfides

Dive	Site	Sample ID	Storage	Used for inocula (temp)
4202	Q vent	4205-5 sulfide	-80ºC, 4ºC	Y (65ºC)
4202	Q vent	4205-5 Alvinella tubes	-80ºC, RNAlater	Y (28ºC)
4203	P vent	4203-3	4ºC	Y (65ºC, 28ºC)

4205	L vent	4205-2	-80ºC, 4ºC	Y (65ºC, 28ºC)
4205	A vent	4205-4	-80ºC, 4ºC	Y (65ºC)
4207	Io vent	4207-4	RNA later	N

Filtered samples

Dive	Sample type	Storage	Used for Inocula
4203	Diffuse flow	-80ºC, RNAlater, 4ºC	Y (28ºC, room temp)
4204	Mud slurp	-80ºC, RNA later	N
4204	Ambient seawater	-80ºC	N
4205	Black filaments	-80ºC	N
4206	Diffuse flow	-80ºC, 4ºC	Y (65ºC)
4207	Diffuse flow, piece of filter obtained with large volume pump (Sievert)	RNA later	

Samples for RNA work, DNA work, and later inoculations were stored using RNAlater,

Deployments

- 3 stainless steel mesh cultivators cultivators were deployed in diffuse hydrothermal flows at Marker 8/11 area

Inoculations

Positive enrichments

Dive	Sample type (ID)	Medium/Temperatue	# of transfers	Notes
4202	Sulfide alvinella tube (4202-5)	Nitrate reducing/28ºC	3	
4202	Sulfide alvinella tube (4202-5)	Aerobic Thiosulfate oxidizing/28ºC	3	Acid production
4202	Sulfide (4202-5)	Nitrate reducing/65ºC	1	
4202	Sulfide (4202-5)	Anaerobic Thiosulfate oxidizing/65ºC	1	
4203	Sulfide (4203-3)	Nitrate reducing/65ºC	3	
4203	Sulfide (4203-3)	Aerobic Thiosulfate oxidizing/28ºC	1	Base production
4203	Diffuse flow (major 20)	Aerobic Thiosulfate oxidizing/28ºC	3	Acid production
4203	Diffuse flow (major 20)	Aerobic Thiosulfate oxidizing/room temp	2	Acid production

4205	Sulfide (4205-2)	Aerobic Thiosulfate oxidizing/28ºC	1	Base production
4206	Diffuse flow (major 04)	Nitrate reducing/65ºC	3	EPS production

Headspace gas composition for nitrate reducing and anaerobic thiosulfate oxidizing media was H2/CO2 (80:20).

Future Work

Sulfides and Fluid samples (Rutgers laboratory)
-Extraction of DNA and RNA from samples stored, respectively, under -80ºC and RNAlater. DNA: Survey of functional genes, nitrate reducers and for methanogens/methanotrophs. RNA: Look for RNA transcripts of functional genes (nitrate reduction).
-Inoculation of sulfide and fluid samples at Rutgers lab for isolation, identification, and characterization of organisms from cultures positive for growth and for MPN work.

Inoculations (Rutgers laboratory)
-Isolation, identification, and characterization of organisms from cultures positive for growth.

Deployments (R/V Atalntis/Rutgers laboratory)
-Retrieval of stainless steel mesh cultivators in Jan 2007. Collection, examination, and storage of biomass from cultivators for RNA and DNA work as well as for inoculation.

Sievert, Taylor, Summons, Molyneaux, Sylva (WHOI, MIT)

Work during this cruise was carried out under the auspices of the NSF grant "Collaborative Research: Microbiology and Biogeochemistry of Autotrophic Microbes in the Subsurface at Hydrothermal Vents: Filamentous Sulfur Producing Bacteria". The recent discovery of microbial populations beneath the deep ocean floor has far reaching implications in biology and has a potentially strong influence on a variety of biogeochemical processes. Presently, the subseafloor biosphere is a poorly defined component of hydrothermal systems; we need better constraints on the nature and extent of this ecosystem and its contribution to primary production at hydrothermal vents.
Our studies entail an integrated microbiological and geochemical study of the abundance, distribution, and diversity of filamentous-S producing microbes (Arcobacter), and an assessment of their ecological role at 9˚N EPR. These microbes have been identified to have contributed to the production of white flocculent material that was discharged in great amounts from so-called "blizzard" or "snowblower" vents in response to the eruption at 9°N on the East Pacific Rise in 1991. The recent eruptive event at 9°N EPR with possible output of subsurface biomass is perfectly suited to further our understanding of this peculiar process. Our studies are designed to test the following general hypotheses:

1) **The prime habitat of filamentous-S-forming microbes is the shallow subsurface, and snowblower vents represent a snapshot sample of that persistent biosphere.**
2) **A significant portion of CO_2-fixation in the subsurface is carried out by epsilon proteobacteria related to *Candidatus* Arcobacter sulfidicus by means of the reductive TCA cycle.**
3) **The organisms forming filamentous-S produce a distinctive geochemical signature (biomarker, isotope) that is deposited and may eventually become part of the geological record.**

During this cruise we were particularly interested in finding and sampling so-called snowblower vents, which were a characteristic feature subsequent to the eruption in 1991. However, although the water in the ASCT was milky and also contained flocculent material, we were not able to find any snowblower vent during the duration of the cruise. This could be either attributed to the possibility that the phase of intense snowblower activity has ceased or to the limited number of dives we had available to survey the area. In lieu of a snowblower vent, we identified a diffuse vent site for more detailed studies. This site lies within the former Marker 82 area and was marked with a new marker #8 (x:4608, y: 77581, Hdg 310, Depth 2503). Extensive diffuse flow was observed at this site with temperatures ranging between 10 and 30˚C. New lava flow covered the area and in areas of diffuse flow the new basalt was covered with white staining, in particular the underside of collected basalt samples. In addition, tubeworms have already started colonizing the underside of these rocks. As a first step to address the above stated hypotheses, the following approaches were pursued:

- **Cultivation of microaerophilic, chemoautotrophic microorganisms from diffuse flow.** Fluid samples were obtained with majors. Different media were inoculated and dilution series performed. Enrichments were incubated at a variety of ranging between 20 and 70˚C.
- **Deployment of ArcoTrap colonization devices to initiate growth of filamentous sulfur forming microbes.** However, no extensive colonization observed at sited deployed, possibly due to short duration of deployment (4 days) and, more likely, relatively low temperature of the diffuse flow (10-17˚C). However, a very fine white film formed on the inside and outside of one of the colonization devices. Swabs of this material contained amplifiable DNA which should reveal which organisms are early colonizers of this diffuse flow. Small limpets were found on the colonizer that developed the biofilm and were provided to Tim Shank's group.
- **Incubation with ^{13}C bicarbonate to identify autotrophic microbes.** Water from diffuse flow sampled with two pairs of majors (~1.2 l; additional ~900 ml were filtered for further analyses in Vetriani's lab, ~130 ml from each bottle was used for chemical analyses by vonDamm's group, 50 ml each were used for cultivation for Vetriani's and Sievert's group, and 50 ml was used for chemical analyses by Luther's group) was incubated at two different temperatures (31˚C and 50˚C) in the presence of ^{13}C-labeled bicarbonate for a total duration of seven days. A variety of electron acceptor additions and head space gas phases were added to

stimulate growth of autotrophic microbes. Subsamples were taken for lipid biomarker and nucleic acid analyses.

- **Filtering of water with large volume pump in situ to obtain large amounts of biomass for lipid biomarker analyses and molecular biological work.** We had one successful deployment and operation of large volume McLane pump. The device was transported to and from the bottom via elevator and transported to the site of sampling by Alvin. The pump intake nozzle was placed directly into the fairly vigorous diffuse flow emanating from the vent. A small temperature logger at the tip of the intake nozzle recorded temperature before, during and after sampling. The 1 min interval temperature record revealed a high frequency variability of ~5°C with a mean temperature that ranged between 13 and 16°C. The slowly varying average temperature appeared to be a tidal variation in the temperature record, though correlation with tides at 9˚N will be necessary to confirm this. During filter sampling there was no indication of significant seawater intrusion (as would be evidenced by an abrupt lowering of the temperature recorded during filtration by the sensor at the tip of the intake). During deployment the pump filtered ~1400 l of vent fluid, resulting in enough biomass for detailed molecular biological and organic geochemical analyses. A quarter of the filter was fixed in RNA-Later and given to Vetriani to look at gene expression; the remainder was frozen at -80°C for subsequent molecular analysis. Initially we had planned for at least two deployments of the pump, but because of time and logistical constraints this was not possible. In the future, it is planned to obtain more large volume samples at different vents to further our understanding of the microbial communities living in the subseafloor.

In addition to these primary objectives, we were also interested in studying the colonization of newly exposed surfaces at vents by microbes and the interactions between microbes and the colonization by invertebrates, the latter in collaboration with Tim Shank (WHOI). For this we sampled basalt that had signs of microbial colonization (samples 4203-5, 4203-6, 4205-3, 4207-3). Subsamples were taken to study the composition and diversity of microbes potentially colonizing the basalt at diffuse flow areas. We observed extensive white staining, possibly of microbial origin. Samples were prepared for subsequent nucleic acid extraction as well as fluorescence in situ hybridization (FISH).

Furthermore, we sampled chimneys in collaboration with Rachel Haymon (UCSB) to correlate distribution and diversity of microbes in chimneys with mineralogy. For this we obtained chimneys which were first subsampled for microbiology (cultivation, DNA/RNA extraction, FISH) and subsequently dried for further mineralogical analyses. The samples obtained for later analyses are: 4202-3, 4202-5, 4203-3, 4203-7, 4205-2, 4205-4, and 4207-4.

Preliminary results

Cultivation

In total 160 tubes were inoculated and incubated at 4 different temperatures: 2 @ 20°, 43 @ 30°C, 47 @ 50°, 17 @ 65°C, and 51 @ 70°C

So far the following enrichements were successful (as of 7/2/06, 10:00 am)

Dive	Sulfur Source	Headspace	Sample	Incub T (°C)
4202	S_2O_3	N/C/O/H	sulfide	70
4202	S_2O_3	N/C/O/H	*Alvinella* tube	70
4203	S_2O_3	N/C/O	Majors water	30
4205	none	H/C/O	sulfide	50
4205	S_2O_3	N/C/O/H	sulfide	70
4205	S_2O_3	N/C/O/H	*Alvinella* tube	70
4206	S_2O_3	N/C/O	water from majors	30

Headspace key:
N/C/O/H = 80% N_2/20% + CO_2 + 20% air in headspace + H_2 overpressure
N/C/O = 80% N_2/20% CO_2 + 20% air in headspace + 80% N_2/20% CO_2 overpressure
H/C/O = 80% H/20% CO_2 + 20% air in headspace + H_2 overpressure
N/C = 80% N_2/20% CO_2

DNA extraction/PCR

DNA was successfully extracted on the ship from chips of glass possessing the "white stain" indicating possible microbial origin. Subsequent PCR further confirmed that biofilm is composed of bacteria, and that archaea are absent. Furthermore, DNA was successfully extracted from swabs of the inner side of one of the ArcoTrap colonization device put out for the collection of filamentous-sulfur forming microbes. Based on subsequent PCR, the thin white film appears to be of bacterial origin. It will be interesting to see how similar or different this biofilm is to the one observed on new basalt.

Future Work in the Laboratory

Future analyses will include the extraction of nucleic acids and lipids from the various samples and subsequent analyses to characterize the microbial communities. We will complement 16 rRNA based surveys for general diversity assessments with the analyses of specific functional genes, e.g. for autotrophic carbon fixation. Determining which organisms have taken up ^{13}C labeled bicarbonate in our incubations will provide important information on which organisms are responsible for autotrophic carbon fixation in situ. In addition, attempts will be made to isolate and subsequently characterize microorganisms from enrichments, also providing information on autotrophic microorganisms inhabiting this system. Rachel Haymon will perform mineralogical analyses on the sampled and dried chimneys. These data will then be correlated with data obtained by our and Vetriani's group.